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BIODIVERSITY RESEARCH

Invasiveness in introduced Australian acacias: the role of species traits and genome size

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ABSTRACT

Aim To assess associations between invasiveness, genome size and species traits in Australian *Acacia* species introduced outside their native range.

Location Global.

Methods Holoploid genome size was determined by flow cytometry for 92 species in *Acacia* subgenus *Phyllodineae* native to Australia. These data were used to test whether genome size was significantly different for invasive ($n = 21$; species known to be established and spreading in foreign environments) and non-invasive ($n = 71$; species that have been introduced to foreign environments but which are not known to be spreading) species. Data for five functional traits [seed mass, specific leaf area (SLA), relative growth rate (RGR), maximum height and dispersal mode] and three characteristics of native range (size, temperature range and precipitation range) were used to test for univariate and multivariate relationships between (1) invasiveness and traits and (2) genome size and traits.

Results Genome size ranged from 1.20 to 2.13 pg/2C and was not significantly smaller in invasive compared with non-invasive acacias. However, invasive acacias were found to be taller and possess a larger native range size and a wider range of annual precipitation when compared with non-invasive acacias. Multivariate analyses revealed significant differences between invasive and non-invasive acacias (PERMANOVA; $P < 0.01$) driven largely by differences in native range characteristics. We detected a positive relationship between genome size and SLA ($P = 0.02$) and elaiosome dispersal mode ($P < 0.01$) in analyses across species, but these findings were not supported by evolutionary divergence analyses.

Main conclusions Genome size variation does not underpin variation in traits associated with the invasive/non-invasive dichotomy in introduced acacias, probably because of the very small DNA values in the *Acacia* species studied. Acacias introduced into new environments are most likely to become invasive if they are tall shrubs or trees and are widely distributed in their native range.

Keywords

***Acacia*, biological invasions, dispersal mode, flow cytometry, genome size, invasive species, maximum height, nuclear DNA content, seed mass, specific leaf area.**

INTRODUCTION

Determining which factors underpin invasiveness is an enduring theme in ecological research. The spread of invasive plant species has previously been linked to introduction effort and residence time (Lockwood *et al.*, 2005; Wilson *et al.*, 2007; Pyšek *et al.*, 2009a) and the history of disturbance at the invaded site (Larson *et al.*, 2001; Lake & Leishman, 2004). However, the attributes of invading species themselves have also emerged as important factors in successful plant invasions

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(Daehler, 2003; Pyšek & Richardson, 2007; van Kleunen *et al.*, 2010). Cytological traits such as ploidy level (Pyšek *et al.*, 2009b) and genome size (Grotkopp *et al.*, 2004; Kubešová *et al.*, 2010), as well as whole plant- and species-level traits, such as specific leaf area (SLA) (van Kleunen *et al.*, 2010) and native range size (Cadotte *et al.*, 2006), have all been implicated in conferring invasion success.

Genome size (or nuclear DNA content) varies more than 2300-fold (0.065–152.23 pg DNA/1C; Greilhuber *et al.*, 2006; Pellicer *et al.*, 2010) across the angiosperm flora, yet the functional consequences of this variation are poorly understood. However, nuclear DNA content has been linked to fundamental processes at different levels of biological complexity (Knight & Beaulieu, 2008). For instance, at the cellular-level, genome size has been positively correlated with the speed of the mitotic cell cycle and with the size of cells and their constituent organelles (Cavalier-Smith, 1978). Genome size is also correlated with ecologically important traits at the whole plant level such as plant longevity (Knight & Ackerly, 2002), seed mass (Thompson, 1990; Wakamiya *et al.*, 1993; Knight & Ackerly, 2002; Grotkopp *et al.*, 2004; Beaulieu *et al.*, 2007a), leaf mass per area and leaf longevity (Morgan & Westoby, 2005). In addition, genome size has been linked with characteristics such as frost tolerance (MacGillivray & Grime, 1995) and tolerance of low annual rainfall and temperature extremes (Knight & Ackerly, 2002). Although the mechanistic explanations for these relationships remain poorly understood, it is thought that the strong and consistent relationships across species and studies indicate that DNA content plays a role in phenotypic expression (i.e. the nucleotypic role of the DNA; Gregory, 2001).

There is some evidence that small genome size may be associated with invasiveness among plant species (Rejmánek *et al.*, 2005). Grotkopp *et al.* (2004) showed a negative relationship between genome size and invasiveness for pines (*Pinus* spp.), possibly due to correlations between seed mass and both genome size and invasiveness. Knight & Ackerly (2002), in a comparative study of 401 Californian plant species, found that exotic species had smaller genome sizes than native species, and Kubešová *et al.* (2010) showed that emerging invaders in the Czech flora possessed smaller genomes than their non-invasive counterparts. However, it is still unclear why these trends occur, in particular whether and how genome size influences more ecologically apparent traits that are functionally implicated in biological invasions (Rejmánek, 1996; Bennett *et al.*, 1998).

In this study, we present the first comprehensive test of how genome size and species trait variation influence the performance of 'Australian acacias', as introduced species around the world. By 'Australian acacias' we mean species in *Acacia* subgenus *Phyllodineae* native to Australia (see Miller *et al.*, 2011; Richardson *et al.*, 2011 for details of this group). In this study, we are particularly interested in the contrast between 'invasive' and 'non-invasive' species. We apply a binary categorization where 'invasive' species are those that are known to have established and spread from sites of introduction in foreign environments (following the criteria set out by Pyšek *et al.*, 2004). 'Non-invasive' species are those that are not known to have spread in foreign environments. A number of Australian acacias have become highly invasive across the world, yet the traits that underpin invasiveness in this group remain largely uninvestigated. We employ a new data set of genome sizes for introduced acacias derived from standardized flow cytometry protocols to ask whether traits related to invasiveness are mediated by genome size. We used a combination of cross-species univariate and multivariate analyses, and phylogenetically independent contrasts to ask whether, on average: (1) invasive acacias have smaller genome sizes than do non-invasive acacias; (2) invasive and non-invasive acacias possess different functional traits and native range characteristics; and (3) the functional traits and range characteristics of acacias are associated with genome size variation. We focused on two reproductive traits (seed mass and dispersal mode), three growth traits [SLA, maximum height and RGR] and three native range characteristics (range size, variation in mean annual temperatures and in mean annual precipitation). The hypothesized relationships between genome size, traits and invasiveness are illustrated in Fig. 1 and described below.

Genome size and invasiveness

We hypothesized that there would be a negative relationship between holoploid genome size and invasiveness, with species classed as invasive having lower 2C DNA content, on average, than those classified as non-invasive (Fig. 1).

Reproductive traits (seed mass and dispersal mode)

We propose two hypotheses about the regenerative traits of introduced acacias: (1) there will be a negative relationship between invasiveness and seed mass (i.e. for a given allocation to reproduction, plants can invest in fewer large seeds or more small seeds; more small seeds provide more colonization opportunities) and (2) invasive acacias will have a greater capacity for long-distance dispersal owing to a higher proportion of species possessing arils than do non-invasive acacias (i.e. acacia seeds possess either an aril that is an adaptation for dispersal by birds or an elaiosome that is an adaptation for dispersal by ants; Fig. 1). In addition, we expect a positive relationship between genome size and seed mass as identified in previous studies (see Knight & Ackerly, 2002 for a list of studies that found a positive correlation between seed mass and genome size; Fig. 1). Finally, we predict no relationship between dispersal mode and genome size (Fig. 1).

Growth traits (specific leaf area, relative growth rate and maximum height)

We hypothesized that (1) invasive Australian *Acacia* species will have greater SLA, RGR and maximum height at maturity than do non-invasive *Acacia* species and (2) genome size will

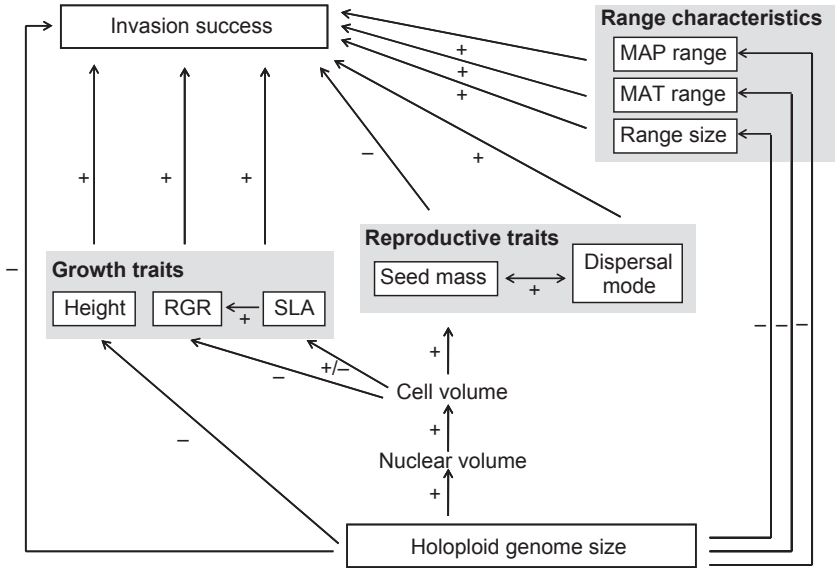

Figure 1 Path diagram of hypothesized relationships between genome size, functional traits and range characteristics and invasiveness in Australian *Acacia* species. + and − signs indicate the hypothesized relationships between variables, and the direction of assumed causal relationships is indicated by arrows. Correlations not statistically tested in our study (e.g. between seed mass and SLA) are not indicated. RGR, relative growth rate; SLA, specific leaf area; MAT, mean annual temperature in the native range; MAP, mean annual precipitation in the native range.

be either positively or negatively correlated with SLA, but will be negatively correlated with RGR and maximum height (Fig. 1). SLA (leaf area per unit dry mass) is a measure of the cost, in terms of dry mass, of deploying photosynthetically active leaf surfaces and is a central component of a species' RGR (Wright *et al.*, 2004). Faster RGR and higher SLA may allow for fast growth of invasive species, which contributes to their invasive success (Grotkopp & Rejmánek, 2007; Leishman *et al.*, 2010; van Kleunen *et al.*, 2010). Larger genome size is associated with larger-volume cells that divide at a slower rate, which may result in a lower RGR (Grotkopp *et al.*, 2004; Francis *et al.*, 2008). As RGR and SLA are positively associated in plants, this should lead to a negative correlation between SLA and genome size; however, this finding has not been consistently supported in previous studies. Maximum height at maturity is also a key determinant of a species' ability to compete for limiting resources, such as light (Falster & Westoby, 2003). Previous studies have identified a negative correlation between plant height and genome size in the angiosperms; however, the mechanistic basis of this relationship remains poorly understood (Knight & Beaulieu, 2008). To test the generality of this finding, we tested for a negative relationship between maximum height at maturity and genome size in introduced acacias.

Range characteristics (range size, mean annual temperature range and mean annual precipitation range)

We tested the following hypotheses (1) invasive acacias will have a larger range size and range of mean annual temperature and mean annual precipitation in their native range than do non-invasive acacias and (2) acacias with larger range sizes and mean annual temperature and precipitation ranges will have smaller genomes. Range size is an integrated macroecological measure of the strength of biotic interactions, and the influence of abiotic factors on shaping the niche and the functional traits of species (Gaston, 2003). The degree of temperature and rainfall variation across a species' range is correlated with range size and is an indication of its climatic tolerance. Species with large genomes have previously shown to be progressively excluded from habitats with extreme environmental conditions (Knight *et al.*, 2005; Temsch *et al.*, 2010), and we propose that extreme abiotic factors are more likely to be encountered across large spatial scales and will lead to genome size reductions in species with large ranges.

METHODS

Species selection

We initially compiled a list of 145 Australian *Acacia* species that have been introduced outside their native range. This list was generated by combining data on Australian acacias from four sources: (1) all species that are present in South African Herbaria (H. Glen, pers. comm.), (2) all species listed in the Global Compendium of Weeds (Randall, 2007), (3) all species introduced to two or more regions throughout the world as presented in Castro-Díez *et al.* (2011) and (4) all species listed as invasive in Richardson *et al.* (2011) (see Appendix S1 in Richardson *et al.* 2011 for a database of sources). Analyses were performed on a subset of 92 *Acacia* species for which genome size data were available. Species were categorized as either invasive (21 species) or non-invasive (71 species), based on Richardson & Rejmánek

(2011) (see Appendix S1 in Supporting Information). Richardson & Rejmánek (2011) define 23 Australia acacias as invasive, but we were unable to obtain reliable genome size estimates for *A. holosericea* A. Cunn. ex G. Don and *A. saligna* (Labill.) H.L. Wendl. and omitted these species from this study.

Genome size data

We follow the genome size nomenclature presented by Greilhuber *et al.* (2005). We use the term 'genome size' and 'holoploid genome size' interchangeably throughout to denote 'holoploid genome size' (expressed in 2C-values), which specifically refer to measurements of DNA content of the whole chromosome complement irrespective of the ploidy level. Although the majority of acacias are likely to be diploid (2n = 26), other ploidy levels have previously been reported (see Plant DNA C-values Database http://data.kew.org/cvalues/). All genome size measurements were made within a single laboratory using standardized protocols, which significantly reduces the likelihood of measurement error influencing results. Holoploid genome sizes were determined for fresh field-collected leaf tissue by propidium iodide flow cytometry using Partec CyFlow SL instrument (Partec GmbH, Münster, Germany) equipped with green diode-pumped solid-state laser (532 nm, 100 mW power output; Cobolt Samba, Solna, Sweden). Sample preparation followed a simplified two-step protocol using Otto I + II buffers as described by Doležel *et al.* (2007). *Bellis perennis* (2C = 3.38 pg; Schönswetter *et al.*, 2007) was selected as an appropriate internal reference standard (with genome size close to but not overlapping with that of acacia samples). Fluorescence intensity of 5000 particles was recorded in each acquisition. Only histograms with peaks of approximately the same height and coefficients of variation below 5% were considered. Each plant was re-analysed at least three times on different days to minimize potential random instrumental drift. If between-day variation (max/min value) exceeded 2%, the outlying value was discarded and the sample remeasured.

Functional trait and range characteristics data

We collated data for five functional traits (seed mass, dispersal mode, SLA, RGR and maximum height) and three range characteristics (range size, mean annual temperature range and mean annual precipitation range). Data sources and the number of species for which trait data were available are presented in Table 1, and further methods are outlined in Appendix S2.

Table 1 Sources and methods used to derive trait data for 92 Australia *Acacia* species used in this study.

Trait	Units	Source	*n*
Seed mass	mg	Royal Botanic Gardens Kew Seed Information Database (http://data.kew.org/sid/)	21 invasive 67 non-invasive
Dispersal mode	categorical: aril/elaiosome	World Wide Wattle database (http://www.worldwidewattle.com/); Edwards *et al.*(2006)	19 invasive 63 non-invasive
SLA	$mm^2\ mg^{-1}$	Mature, fully expanded leaves were collected in the field, scanned and oven-dried to calculate leaf area per unit dry mass	17 invasive 58 non-invasive
RGR	$mg\ mg_{plant}^{-1}\ day^{-1}$	Seedlings were grown in glasshouses and RGR calculated using the equation $RGR = (\log_e H_2 - \log_e H_1)/T$ where H_2 = plant biomass after 92 days; H_1 = plant biomass after 10 days; T = time between harvests (82 days)	17 invasive 30 non-invasive
Maximum height	m	Harden (2004); World Wide Wattle database (http://www.worldwidewattle.com/); Western Australian Herbarium online database (http://florabase.calm.wa.gov.au/)	19 invasive 68 non-invasive
Range size	sq km	Calculated from georeferenced point occurrences from Australia's Virtual Herbarium (AVH; http://avh.rbg.vic.gov.au/avh/public_query.jsp) following Burgman & Fox's (2003) four-step procedure for calculating the α-hull (see Hui *et al.*, 2011 for further details)	21 invasive 69 non-invasive
Mean annual temperature range	°C	Point occurrences from the AVH matched to an interpolated climate surface for the period 1950–2000 (Worldclim: see Hijmans *et al.*, 2005) at a 5 arc minute resolution (http://www.worldclim.org/download) using the intersect point command in Hawth's Tools in ArcGIS v. 9.2 (ESRI 2006)	21 invasive 70 non-invasive
Mean annual precipitation range	mm	Same method as mean annual temperature range	21 invasive 70 non-invasive

RGR, relative growth rate; SLA, specific leaf area.

Statistical analyses

All univariate analyses were performed using SPSS v. 19.0 (SPSS, Chicago, IL, USA) and considered significant at the 0.05 α level. Multivariate procedures were performed by PRIMER v. 6 using the PERMANOVA+ add-on (Clarke & Gorley, 2006).

Relationships between invasiveness and genome size/species traits

Cross-species variation between invasive and non-invasive species in genome size and continuous species traits (seed mass, maximum height, SLA, RGR, range size, the range of mean annual temperature and range of mean annual precipitation) was assessed using the *F*-statistic in a one-way analysis of variance. All variables, with the exception of RGR, were $\log_{10}$ transformed before analysis to reduce skew in the raw data. Differences in the proportion of species exhibiting aril and elaiosome dispersal modes between invasive and non-invasives were assessed using chi-squared goodness-of-fit test.

To complement univariate tests, we also used permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) to determine whether invasive and non-invasive acacias form statistically distinct clusters in multivariate trait space. We tested for differences in the location of the two groups in multidimensional space using a one-factor (invasiveness) design with two levels (invasive or non-invasive). A similarity matrix based on Gower's distance (see Gower, 1971) was constructed on normalized data for 67 species (16 invasive and 51 non-invasive) for which values for all functional traits and range characteristics were known. We excluded measures of RGR from the multivariate analyses because the smaller number of species for which data were available ($n = 47$) significantly reduced the number of species that could be used for multivariate comparison. Significance values were calculated by permutation through the random reassignment of the factor levels using 9999 permutations of the raw data.

We used a multivariate test for homogeneity of dispersion (PERMDISP; Anderson *et al.*, 2006) to identify whether statistical differences in invasive and non-invasive species groups arise from differences in the spread of points around their central point, rather than their location in multivariate space. This test is analogous to Levene's test (Schultz, 1985), which is frequently used to explore equality of variance assumptions in univariate ANOVA.

To visualize patterns in the multivariate data in two-dimensional space, we employed principal component analysis (PCA). The eigenvector scores associated with the first two axes were ranked according to their absolute value, and the two largest scores were used to interpret which traits, if any, underpin clustering patterns on principal component axes 1 and 2.

Relationships between genome size and species traits

The association between continuous species trait values and genome size in introduced acacias was assessed using the ordinary least-squares regression. One-way analysis of variance was used to determine whether species with different dispersal modes have significantly different genome sizes.

Correlated evolutionary divergences in genome size and species traits

We used evolutionary divergence analyses to explore the patterns of association between genome size and species traits identified in cross-species analyses in a phylogenetic context. Divergence analyses allow us to assess whether relationships detected in present-day species at the tips of a phylogenetic tree are conserved across the evolutionary history of our sample of invasive and non-invasive acacias. Specifically, we asked whether divergences in species traits and range characteristics were significantly correlated with divergences in genome size throughout the evolutionary radiation of the Australian acacias.

Phylogenetic tree construction followed the protocols outlined by Miller *et al.* (2011). Trees were constructed using DNA sequences from four plastid regions (psbA-trnH intergenic spacer, trnL-F intron and intergenic spacer, rpl32-trnL intergenic spacer and a portion of the matK intron), and Bayesian statistics were used to determine the most parsimonious relationships between species using MrBayes v. 3.1.2 (Ronquist & Huelsenbeck, 2003). DNA sequence data were available for 77 species of the 92 introduced acacias (19 invasives and 58 non-invasives), and separate trees were constructed from these species for each of the eight traits of interest.

Evolutionary divergence analyses were performed using Phylocom v. 4.1 (Webb *et al.*, 2008) using the DOS command line interface. Divergences in species trait values and divergences in genome size were calculated across nodes of the tree using the 'analysis of traits' module (AOT) in Phylocom (v. 4.1) following the methods outlined by Felsenstein (1985). In each analysis, genome size was used as the independent variable with the continuous traits (seed mass, SLA, maximum height, RGR, range size, temperature range and precipitation range) and the binary trait (dispersal mode) as dependent variables. The significance of relationships between divergences in genome size and continuous species traits was assessed using a linear model in SPSS (v. 19). The regression line in these analyses was forced through the origin to account for ambiguity in the direction of the subtraction giving rise to the divergence value (see Garland *et al.*, 1992). For divergences between genome size and dispersal mode, a one-sample *t*-test was performed against the null hypothesis of no change in genome size across divergences.

RESULTS

A path diagram of the relationships between genome size, traits and invasiveness is provided in Fig. 2.

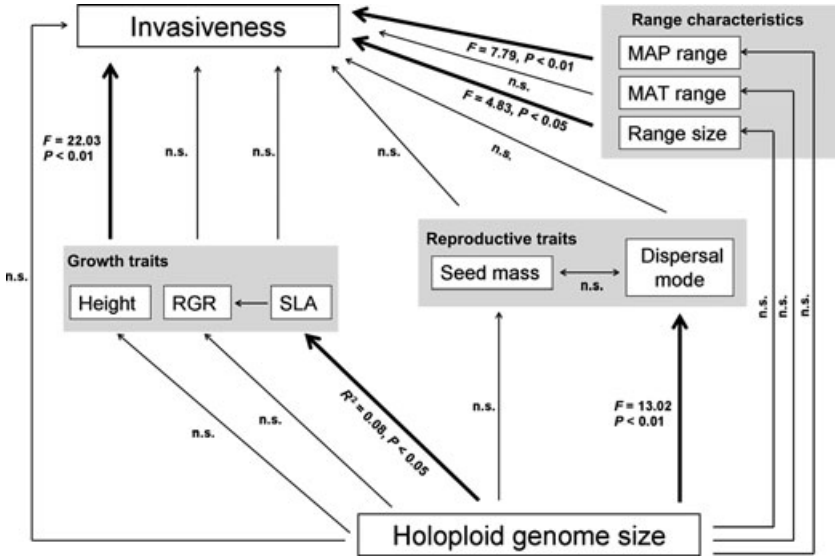

Figure 2 Path diagram describing outcomes of statistical tests of association between genome size, functional traits and range characteristics and invasiveness in Australian *Acacia* species. RGR, relative growth rate; SLA, specific leaf area; MAT, mean annual temperature in the native range; MAP, mean annual precipitation in the native range; n.s., non-significant ($P > 0.05$).

Genome size and invasiveness

Holoploid genome size (2C-values) ranged between 1.20 pg in *A. cupularis* Domin and 2.13 pg in *A. longifolia* (Andrews) Willd. subsp. *longifolia* (Fig. 3). We found no significant difference in holoploid genome size between invasive and non-invasive acacias ($F_{1, 90} = 2.22$, $P = 0.14$; Table 2; invasive mean = 1.60 pg/2C, non-invasive mean = 1.69 pg/2C).

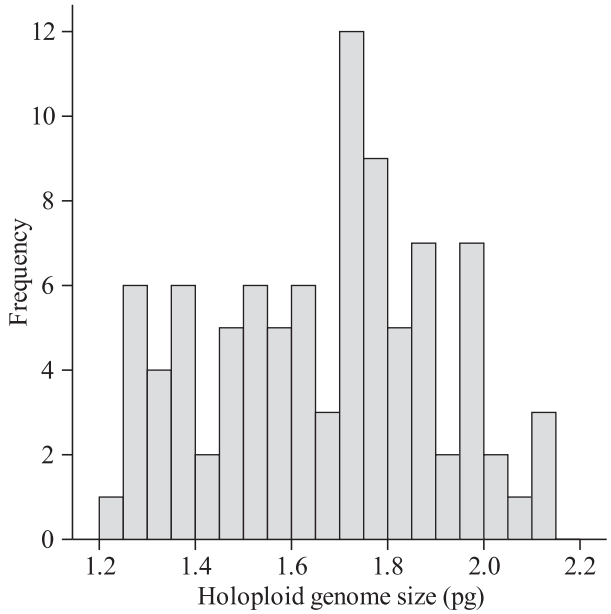

Figure 3 Distribution of holoploid genome size (2C-values) of the 92 Australian *Acacia* species used in this study.

Species traits and invasiveness

Univariate analyses

There were significant differences between invasive and non-invasive acacias in three of the eight species traits examined (Table 2). Maximum height was, on average, 2.2-fold larger in invasive acacias than non-invasive acacias (invasive mean = 12.11 m, non-invasive mean = 5.45 m; $F_{1, 86} = 22.03$, $P < 0.001$; Fig. 4c). Similarly, range size was significantly larger in invasives than in non-invasives (invasive mean =

Table 2 Results of analyses of the association between invasiveness and trait variation in eight traits of introduced Australian *Acacia* species. Significance of relationships was tested using one-way analysis of variance for all traits except dispersal mode that was tested using the chi-squared goodness-of-fit test ($\alpha = 0.05$).

Continuous traits	*n*	*F*	*P*	d.f.
Holoploid genome size	92	2.22	0.14	1, 91
Log (seed mass)	88	0.24	0.63	1, 87
Log (specific leaf area)	75	2.01	0.16	1, 74
Log (maximum height)	87	22.03	< 0.001**	1, 86
Relative growth rate	47	0.25	0.62	1, 46
Log (range size)	90	4.83	0.03*	1, 89
Log (temperature range)	91	0.22	0.64	1, 90
Log (precipitation range)	91	7.79	< 0.01**	1, 90
Binary traits	***n***	**χ^2**	***P***	**d.f.**
Dispersal mode	82	2.94	0.08	1

Significance values: *$P < 0.05$, **$P < 0.01$.

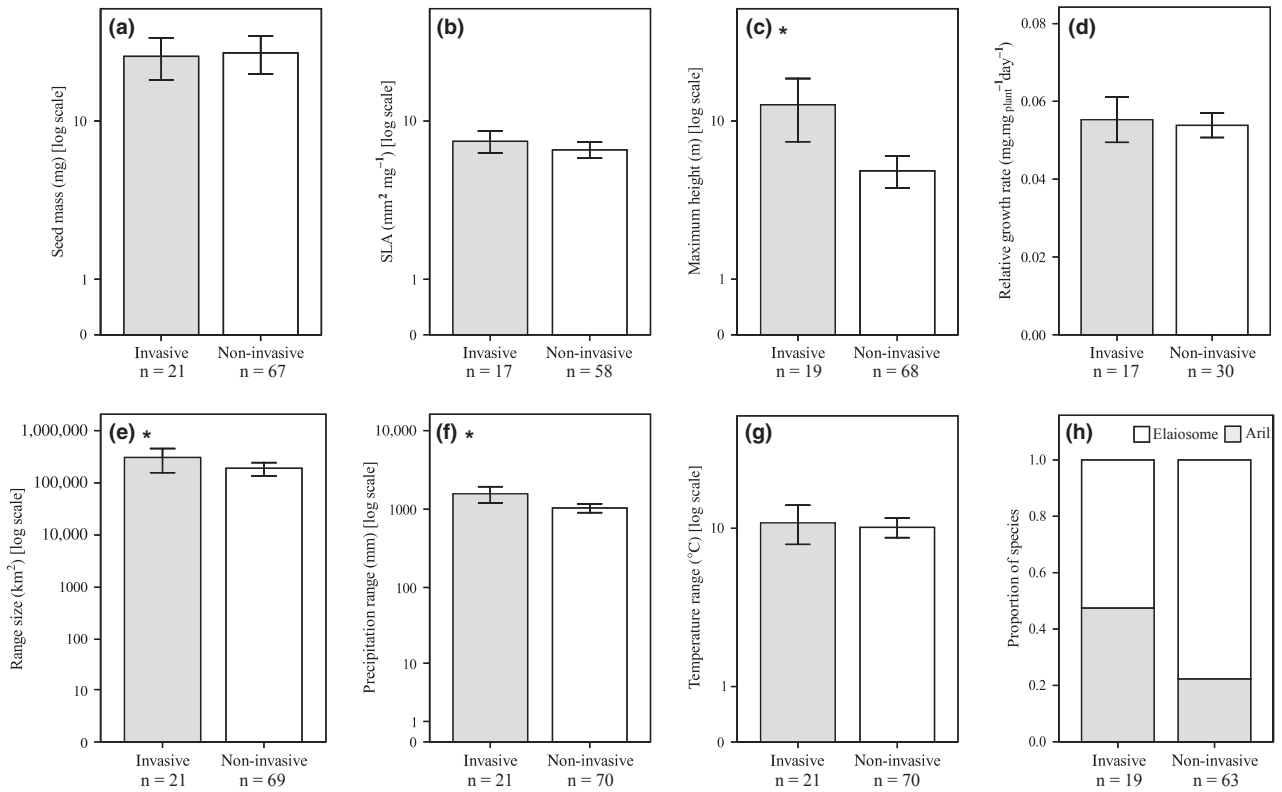

Figure 4 Relationships between invasiveness and eight traits in Australian *Acacia* species: (a) seed mass (mg), (b) specific leaf area (mm^2 mg^{-1}), (c) maximum height (m), (d) relative growth rate (mg mg_{plant}^{-1} day^{-1}), (e) range size (sq km), (f) mean annual temperature range (°C), (g) mean annual precipitation range (mm), and (h) dispersal mode (aril/elaiosome). Columns represent means ± 2 SD. Significance of relationships was tested using one-way analysis of variance for all traits except dispersal mode that was tested using the chi-squared goodness-of-fit test ($\alpha = 0.05$). Asterisks on graphs indicate that significant differences exist between invasive and non-invasive species.

305,735 sq km, non-invasive mean = 190,198 sq km; $F_{1, 89} = 4.83$, $P = 0.03$; Fig. 4e), as was the range of annual precipitation occupied in the native range (invasive mean = 1676 mm, non-invasive mean = 1035 mm; $F_{1, 90} = 7.79$, $P < 0.01$; Fig. 4f).

There were no significant differences in SLA, RGR, seed mass, dispersal mode or the range of annual temperatures occupied in the native range between invasive and non-invasive acacias in our study (SLA: $F_{1, 74} = 2.01$, $P = 0.16$, Fig. 4b; RGR: $F_{1, 46} = 0.25$, $P = 0.62$, Fig. 4d; seed mass: $F_{1, 87} = 0.24$, $P = 0.63$, Fig. 4a; dispersal mode: $\chi^2 = 2.94$, $P = 0.08$, Fig. 4h; range of mean annual temperatures: $F_{1, 90} = 0.22$, $P = 0.64$, Fig. 4g).

Multivariate analyses

We identified highly significant differences between invasive and non-invasive introduced acacias when all species traits, including genome size, were combined in a multivariate analysis of variance (PERMANOVA: pseudo $F_{1, 65} = 6.4$, $P < 0.01$). Bivariate plots of the first two principal component (PC) axis scores indicate separate patterns of clustering for invasive and non-invasive acacias in two-dimensional space (Fig. 5). The first two PC axes explained approximately half (49%) of the total variation in species trait values across the 67 introduced acacias for which data were available. We used a multivariate test of dispersion (PERMDISP) to examine whether differences in the location of data clusters detected using the PERMANOVA approach could be attributed to differences in the dispersion of invasive and non-invasive species groupings in multivariate space. We found no evidence to support this idea (PERMDISP: $F_{1, 65} = 1.29$, $P = 0.31$), which supports our PERMANOVA findings that clusters occupy distinct locations in multivariate space.

The first PC axis separated invasive and non-invasive acacias on the basis of range characteristics (see Table 3). That is, mean annual temperature range and range size had the highest eigenvector scores on axis 1 (−0.59 and −0.52 respectively); however, mean annual precipitation range was also highly weighted on this axis (−0.51). Invasive acacias generally occupied the lower end of PC axis 1, indicating that they possess larger range size and occur across a wider range of annual temperatures in their native range than do non-invasive acacias.

There was no distinct clustering of invasives and non-invasives along PC axis 2 (Fig. 5). This second axis was strongly associated with SLA and seed mass (eigenvector

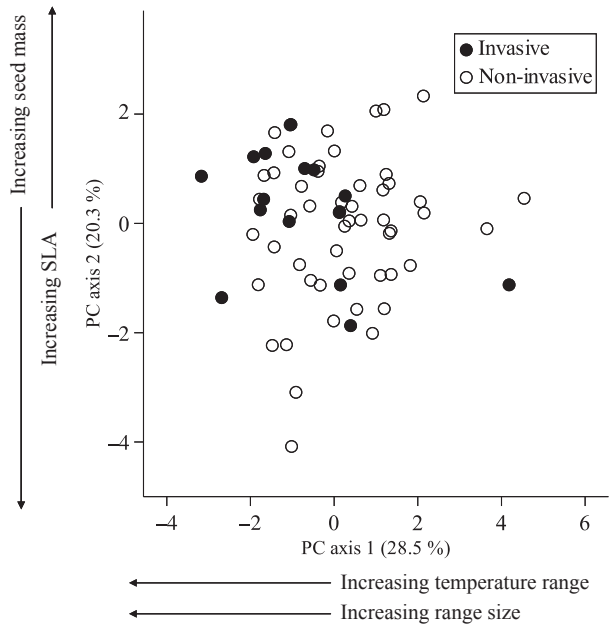

Figure 5 Principal component analysis (PCA) ordination of 67 species of introduced Australian *Acacia* species on the basis of eight traits. Closed circles represent invasive species ($n = 16$) and open circles represent non-invasive species ($n = 51$). Arrows below the figure indicate traits with the highest eigenvector scores on principal component (PC) axes 1 (the *x*-axis) and 2 (the *y*-axis; see Table 3). These traits are ordered such that the trait with the highest eigenvector score appears closest to the PC axis.

Table 3 Eigenvalues associated with the first three principal component axes derived from the eight trait × 67 species matrix of introduced Australian *Acacia* species. Numbers in parentheses represent the total amount of variation explained by each axis. Values are ranked by their absolute value along principal component axis 1. Numbers in bold represent the three highest eigenvector scores along each axis.

Trait	Axis 1 (28.5%)	Axis 2 (20.3%)	Axis 3 (16.8%)
Range of mean annual temperature	**−0.59**	0.03	0.24
Range size	**−0.52**	−0.28	0.14
Range of mean annual precipitation	**−0.51**	**0.36**	−0.04
Maximum height	−0.27	0.28	**−0.35**
Dispersal mode	−0.16	−0.02	**−0.69**
Specific leaf area	0.12	**0.62**	−0.01
Seed mass	−0.11	**−0.46**	0.10
Genome size	0.02	0.33	**0.56**
Eigenvalue	2.28	1.63	1.34

scores: 0.62 and −0.46 respectively; Table 3), and the lack of strong clustering patterns on this axis reflects our univariate results of no significant differences in these two traits between invasive and non-invasive acacias.

Species traits and genome size

Cross-species analyses

Relationships between genome size and species traits were similar between invasive and non-invasive acacias ($P > 0.05$ for all tests; data not shown), and all species were grouped for subsequent analyses (Table 4). We identified a significant positive correlation between holoploid genome size and SLA ($R^2 = 0.08$, d.f. = 1, 74, $P = 0.02$; Fig. 6b) across all introduced acacias in our study. Although this relationship was statistically significant ($P = 0.02$), its explanatory power was low ($R^2 = 0.08$), which may limit its biological relevance. Genome size was significantly larger in acacias with elaiosomes than acacias with arils ($F_{1, 81} = 13.02$, $P < 0.01$; Fig. 6h). Interestingly, we found no evidence of a relationship between dispersal mode and seed mass ($F_{1, 78} = 0.20$, $P = 0.66$), indicating that the strong association between dispersal mode and genome size is not the result of an underlying association with seed mass.

We found no further significant relationships between genome size and the remaining five traits. That is, we did not find negative correlations as hypothesized between genome size and RGR ($R^2 = 0.001$, d.f. = 1, 46, $P = 0.80$; Fig. 6d), maximum height ($R^2 = 0.001$, d.f. = 1, 86, $P = 0.86$; Fig. 6c), range size ($R^2 = 0.02$, d.f. = 1, 89, $P = 0.18$; Fig. 6e), temperature range ($R^2 = 0.01$, d.f. = 1, 90, $P = 0.32$; Fig. 6g) or precipitation range ($R^2 = 0.01$, d.f. = 1, 90, $P = 0.35$; Fig. 6f). We also did not find a positive correlation as expected between genome size and seed mass in introduced Australian acacias ($R^2 = 0.03$, d.f. = 1, 87, $P = 0.14$; Fig. 6a).

Evolutionary divergence analyses

Phylogenetic analyses largely supported the findings of our cross-species analyses of relationships between species traits and genome size in introduced Australian acacias (Table 4). For instance, changes in maximum height across nodes in the acacia phylogeny were not consistently associated with changes in genome size ($R^2 = 0.09$, d.f. = 1, 32, $P = 0.09$; Fig. 7c), which corroborates the findings of our cross-species analysis. Similarly, we found no correlation between evolutionary divergences in genome size and divergences in seed mass, divergences in RGR or divergences in any of the environmental range parameters tested (see Fig. 7 & Table 4), which was consistent with our cross-species analyses.

Divergences in SLA and dispersal mode were not correlated with divergences in genome size across the acacia phylogeny (SLA: $R^2 = 0.03$, d.f. = 1, 40, $P = 0.31$; Fig. 7b; dispersal mode: $t = -1.88$, $P = 0.09$; Fig. 7h), despite being significantly correlated among species at the tips of the phylogeny (see Table 4). The inconsistency between the findings of the cross-species and phylogenetic analyses suggests that although a relationship between genome size and SLA, and genome size and dispersal mode exists in our subset of present-day acacias, this pattern is not reflected in the evolutionary radiation of the genus *Acacia* subgenus *Phyllodineae*.

Table 4 Results of analyses across species and across phylogenetically independent contrasts (PICs) for eight traits of introduced Australian *Acacia* species. In cross-species analyses, continuous traits (seed mass, SLA, maximum height, RGR, range size, mean annual temperature range and mean annual precipitation range) were assessed using one-way analysis of variance, and binary traits (dispersal mode) were tested using chi-square goodness-of-fit test. In phylogenetic analyses, divergences between two continuous traits (e.g. genome size and seed mass) were assessed using linear regression with no intercept value, and divergences between one binary and one continuous trait were assessed using a one-sample *t*-test based on the null hypothesis of no change. Genome size was the independent variable in all analyses.

	Cross-species analyses				Phylogenetic analyses			
Continuous traits	*n*	R^2	*P*	d.f.	+ PICs	− PICs	*P*	d.f.
Log (seed mass)	88	0.03	0.14	1, 87	20	24	0.08	1, 43
Log (specific leaf area)	75	0.08	0.02*	1, 74	21	20	0.31	1, 40
Log (maximum height)	87	0.001	0.86	1, 86	16	17	0.09	1, 32
Log (relative growth rate)	47	0.001	0.80	1, 46	12	15	0.41	1, 26
Log (range size)	90	0.02	0.18	1, 89	21	18	0.57	1, 38
Log (temperature range)	91	0.01	0.32	1, 90	19	20	0.09	1, 38
Log (precipitation range)	91	0.01	0.35	1, 90	19	20	0.50	1, 38
Binary traits	*n*	*F*	*P*	d.f.	*n*	*t*	*P*	d.f.
Dispersal mode	82	13.02	0.001**	1, 81	12	−1.88	0.09	11

Significance values: *$P < 0.05$, **$P < 0.01$.

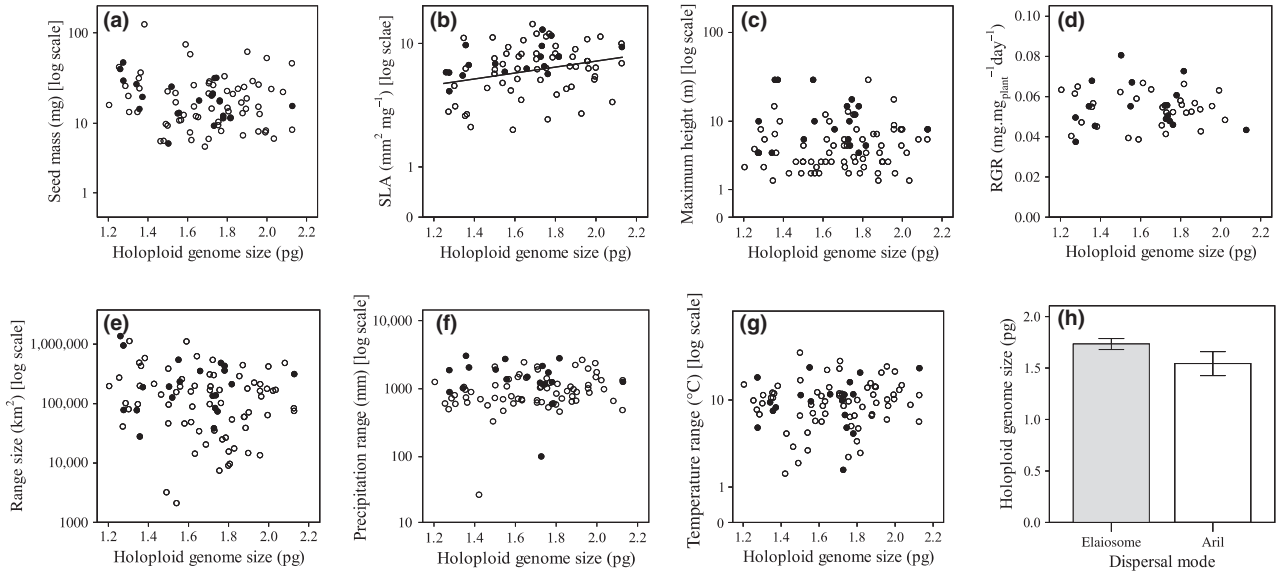

Figure 6 Relationships between holoploid genome size and eight traits of introduced Australian *Acacia* species: (a) seed mass (mg), (b) specific leaf area ($mm^2\ mg^{-1}$), (c) maximum height (m), (d) relative growth rate ($mg\ mg_{plant}^{-1}\ day^{-1}$), (e) range size (sq km), (f) mean annual precipitation range (mm), (g) mean annual temperature range (°C) and (h) dispersal mode (aril/elaiosome). Closed circles represent invasive species, and open circles represent non-invasive species. The line in (b) represents the significant ordinary least-squares regression line between genome size and SLA for all introduced acacias. Significance of relationships was tested using ordinary least-squares regression for all traits except dispersal mode that was tested using one-way analysis of variance ($\alpha = 0.05$).

DISCUSSION

In this study, we used a robust genome size data set to test whether small genome size is associated with invasiveness among a large number of introduced acacias, as has been found in previous within-genus (Grotkopp *et al.*, 2004) and cross-species studies (Knight & Ackerly, 2002; Kubešová *et al.*, 2010). We analysed a comprehensive data set incorporating reproductive, growth and range characteristics to assess whether any relationship between genome size and invasiveness was because of secondary correlations with other traits. We found that invasive acacias do not have smaller genome size on average than non-invasive acacias, although there were significant associations between plant traits and invasiveness, and plant traits and genome size (Figs 4, 6 & 7).

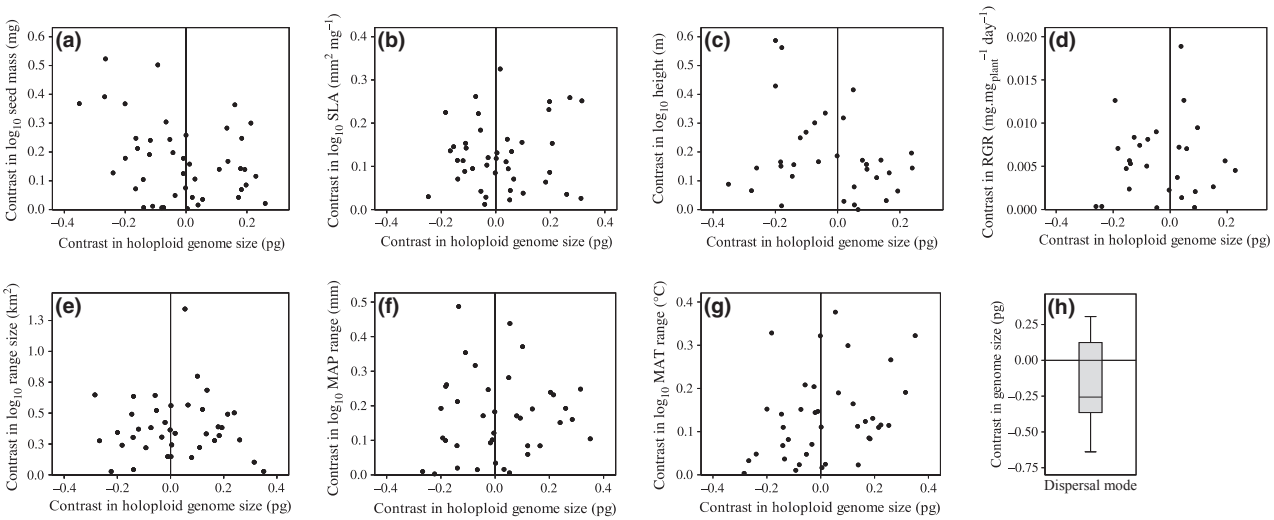

Figure 7 Results of evolutionary divergence analyses of divergences in genome size (independent variable) and divergences in seven continuous and one binary species trait for Australian *Acacia* species: (a) seed mass (mg), (b) specific leaf area (mm^2 mg^{-1}), (c) maximum height (m), (d) relative growth rate (mg mg_{plant}^{-1} day^{-1}), (e) range size (sq km), (f) mean annual temperature range (°C), (g) mean annual precipitation range (mm) and (h) dispersal mode (aril/elaiosome). For dispersal mode (h), the line at the origin represents the null expectation of no change in the trait's value across divergences in the invasion status.

Why is genome size not correlated with invasiveness in Australian acacias?

There are several potential explanations for why previously reported associations between genome size and invasiveness were not found among Australian acacias. First, the relationship between invasiveness and genome size found within the genus *Pinus* (Grotkopp *et al.*, 2004) was attributed to secondary correlations of both traits with seed mass. In our study, we found no relationship between genome size and seed mass. Genome sizes of all acacias in our study (1C = 0.60–1.07 pg) fall into the smallest out of five genome size categories as defined by Leitch *et al.* (1998) and are quite small compared with those among the pines (21.98–37.68 pg DNA/1C; Grotkopp *et al.*, 2004) and to angiosperms generally (0.065–152.23 pg DNA/1C; Greilhuber *et al.*, 2006; Pellicer *et al.*, 2010). Beaulieu *et al.* (2007a) showed that among the angiosperms, species with small genome sizes display a wide range of seed mass, while in contrast among the gymnosperms there is a positive correlation between seed mass and genome size. The lack of a relationship between genome size and seed mass may therefore explain the lack of a relationship between genome size and invasiveness in the acacias.

Second, relationships between genome size and invasiveness may be due to secondary correlations with traits other than seed mass, such as SLA. There are two opposing hypotheses for the relationship between genome size and SLA and RGR. First, small cell volumes and fast division rates associated with small genome sizes will result in a negative relationship between genome size and SLA (Sims & Price, 1985; Grotkopp *et al.*, 2004). Alternatively, large cell types may reduce tissue density by reducing the amount of cell wall per unit volume, resulting in higher SLA (Morgan & Westoby, 2005; Beaulieu *et al.*, 2007b). Large SLA has been shown in a number of studies to be associated with invasiveness (Grotkopp & Rejmánek, 2007; Leishman *et al.*, 2010; van Kleunen *et al.*, 2010), presumably as it confers a capacity for rapid growth when resources are not limiting. In this study, we found that among the acacias, species with large genome sizes had larger SLA, but there was no relationship between SLA and invasiveness, or between RGR and invasiveness. Interestingly, positive relationships between genome size and SLA have been reported within angiosperms (Morgan & Westoby, 2005; Beaulieu *et al.*, 2007b; Knight & Beaulieu, 2008), and negative relationships within gymnosperms (Grotkopp *et al.*, 2004; Morgan & Westoby, 2005). This may also account for the lack of consistent findings between the acacias in this study and the pines studied by Grotkopp *et al.* (2004).

As far as we know, this is the first study to report a correlation between dispersal mode and genome size. We can, however, offer no plausible causative explanation for this relationship and encourage further investigation of its generality. Importantly, we found no significant correlations in cross-species or PICs between dispersal mode and invasiveness despite our expectation that acacias with arils on their seeds, which facilitate long-distance dispersal by birds, were more likely to be invasive than those with elaiosomes.

Finally, genome size may be associated with invasiveness through the characteristics of the native range. We hypothesized that species that had a large native range encompassing a large range in climatic conditions were most likely to encounter harsh environmental conditions, and large genome size appears to be selected against in such conditions (Knight *et al.*, 2005; Temsch *et al.*, 2010). However, although we

found that species with a large native range were more likely to be invasive, we found no relationships between range characteristics and genome size. One potential explanation for this result is that the relatively small average 2C DNA values of acacias in this study are insufficient to pick up signals of exclusion of species with large genome sizes in extreme environmental conditions. Alternately, there may be little or no correspondence between spatial range and the chance of encountering extreme climatic conditions in the Australian landscape.

The potential pathways that could contribute to a relationship between genome size and invasiveness were presented in Fig. 1. It is clear that among Australian acacias, there are a number of significant relationships between traits and invasiveness, and between traits and genome size. However, these relationships do not underpin a direct relationship between invasiveness and genome size (Fig. 2). Acacias with small genome size have a slower growth strategy but have a capacity for long-distance dispersal, while seed mass, RGR, height and range characteristics were not associated with genome size variation. Given the relatively small variation in genome size and other traits such as seed mass, height and SLA among the acacias, perhaps it is not surprising that the relationships between genome size and invasiveness found in previous studies (Knight & Ackerly, 2002; Grotkopp *et al.*, 2004; Kubešová *et al.*, 2010) were not upheld.

Which traits separate invasive from non-invasive introduced Australian acacias?

We found that there were significant trait differences between invasive and non-invasive acacias. Invasive acacias were taller and had larger native range sizes associated with greater variation in climatic conditions. Why might invasive acacias be taller? Taller plants may be better able to out-compete neighbouring plants for limited resources (Holmes & Cowling, 1997; Rejmánek *et al.*, 2005; Moles *et al.*, 2009). In addition, taller species are likely to be favoured in forestry plantings, and thus the greater introduction effort and resultant propagule pressure may explain their relative success compared with shorter species. Similarly, foresters may have favoured species with broader moisture requirements for plantings that increased their cultivation. This study required that all species in the data set had been introduced to at least two different regions; however, we recognize that planting effort may have varied substantially across the species, and we were unable to control for this given the limited data available.

Range size was also shown to differ significantly between invasive and non-invasive acacias, consistent with findings for other plant groups (Rejmánek *et al.*, 2005). Large native ranges may contribute to invasiveness through several processes: larger range size may be associated with tolerance of a wide range of environmental conditions, with the capacity to disperse across the landscape and with high genetic variation (see discussion in Hui *et al.*, 2011).

Specific leaf area is a plant trait that has shown to be associated with invasive success across a broad range of species (see Grotkopp & Rejmánek, 2007; Leishman *et al.*, 2010; van Kleunen *et al.*, 2010), but we did not find this for the acacias. Similarly, Grotkopp *et al.* (2010) also found no relationship between invasiveness and SLA across a range of angiosperms and attributed this finding to differing physiological and biomass allocation patterns among species. Acacias have been widely introduced for the timber trade, fuel wood production and revegetation of degraded sites, and traits associated with rapid growth have been highly favoured during species selection (Kull *et al.*, 2011). We propose that the lack of significant difference in SLA between invasives and non-invasives is the result of a bias towards the introduction of fast-growing species and that this underscores the importance of introduction history in determining invasiveness (see for example Blackburn & Duncan, 2001). We suggest that a stronger signal may be detected if we were to compare acacias that have been widely introduced with those never introduced outside their native range.

Previous studies have shown that the capacity for long-distance dispersal is important for successful invaders (Trakhtenbrot *et al.*, 2005) and that invasives are often more likely to have wind- or vertebrate-dispersed seeds compared with co-occurring natives or non-invasive species (Higgins & Richardson, 1999; Clark *et al.*, 2001; Wilson *et al.*, 2009). In particular, species with vertebrate-dispersed seeds are recognized as particularly successful invaders (Rejmánek *et al.*, 2005), and few successful invaders have seeds adapted for dispersal by invertebrates such as ants (see Lake & Leishman, 2004). However, our study shows that dispersal mode is not a significant factor in determining which Australian acacias have become invasive outside their native range, which may point to the role of human-mediated dispersal as a critical determinant of spread in this genus.

The results of multivariate analyses were broadly consistent with the univariate results, confirming the importance of large range size encompassing wide climatic variation for invasiveness. However, while the univariate analyses showed that taller acacias were more likely to be invasive, the multivariate analysis suggested that larger SLA and smaller seed mass were the most important traits associated with invasiveness. This slight discrepancy may be due to the reduced data set available for the multivariate analyses or it may highlight the presence of a spurious correlation between temperature and rainfall in our data set. Importantly, however, genome size was not associated with the primary axes describing variation among the introduced acacias.

This study has shown that acacias introduced into new environments are most likely to become invasive if they are tall shrubs or trees and are widely distributed in their native range. However, we recognize that the ability of species to become invasive may be strongly linked to introduction effort and to management in the introduction area (Griffin *et al.*, 2011). Thus, the lack of comprehensive, standardized data on introduction effort for Australian acacias may limit our ability to

predict which species may become invasive in the future. However, given the high productive value of acacias globally, and the continuing search for new species to use for wood and fuel production in many regions, it is critical that factors that predict invasion success are included in risk assessment frameworks.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Species, their invasion status and trait data for 92 Australian acacias.

Appendix S2 Methods of calculation for RGR, SLA, seed mass and range characteristics used in this study.

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